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Karyotype characterization of planthopper species *Hysteropterum albaceticum* Dlabola, 1983 and *Agalmatium bilobum* (Fieber, 1877) (Homoptera: Auchenorrhyncha: Issidae) using AgNOR-, C- and DAPI/CMA$_3$ -banding techniques

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Abstract. Males of *Hysteropterum albaceticum* Dlabola, 1983 and *Agalmatium bilobum* (Fieber, 1877) display a chromosomal complement of 2n = 26 + X, which is a basic one of the tribe Issini (Issidae). In the present study, silver staining, C-banding and a base specific CMA$_3$ -and DAPI-banding were used with the aim of identifying possible cytogenetic markers and distinguishing between karyotypes with the same chromosome number and no detectable inter-species differences in karyotype structure. We characterized the species studied in terms of the distribution and molecular structure of C-heterochromatin regions and the location of nucleolus organizer regions (NORs). The species are shown to differ considerably in the amount of heterochromatin, its distribution pattern along the karyotypes and its stain ability with DAPI and CMA$_3$.

Key words: *Hysteropterum albaceticum*, *Agalmatium bilobum*, Homoptera, Auchenorrhyncha, Issidae, NORs, C-heterochromatin, DAPI/CMA$_3$ -staining, chiasmata, polymorphism.

Introduction

The chromosomes of Auchenorrhyncha, as well as of all other Homoptera groups, are holokinetic (Halkka, 1959). These chromosomes are characterized by the lack of a defined centromeric locus (localized centromere), which makes the identification of individual chromosomal pairs and detection of chromosomal rearrangements in karyotypes very difficult. Most cytogenetic studies on the Auchenorrhyncha were performed using standard chromosome staining techniques. The exceptions are the investigation of *Tibicen bihamatus* (Motschulsky, 1861) and *Platypleura kuroiwai* Matsumura, 1917 (the family Cicadidae) involving C-banding (Perepelov et al., 2002), and that of *Philaenus spumarius* (Linnaeus, 1758) and *Ph. arslani* Abdul-Nour & Lahoud, 1996 (Aphrophoridae) performed by means of a number of differential staining techniques (Kuznetsova et al., 2003; Maryańska-Nadachowska et al., 2008). The techniques applied characteristically highlight specific chromosomal regions, such as

the nucleolus organizer regions (NORs) and C- heterochromatin blocks containing late-replicating repetitive DNA. Moreover, in both studies on the *Philaenus* Stål, 1864, the DNA binding fluorochromes CMA_3 and DAPI were used to reveal whether the heterochromatin is enriched in GC or AT bases. The above methodologies have provided cytogenetic markers for understanding chromosome diversity and suggesting mechanisms of chromosome rearrangements in the genus *Philaenus* (Kuznetsova et al., 2003; Maryańska-Nadachowska et al., 2008).

The currently available cytogenetic evidence on the world-wide planthopper family Issidae Spinola, 1839 (Fulgoroidea) is restricted to 22 species and 15 genera, all but one species belonging to the tribe Issini Spinola, 1839 (see Maryańska-Nadachowska et al., 2006 and references therein). It is generally assumed that holokinetic chromosomes facilitate karyotype evolution through fission and fusion of chromosomes (White, 1973), however the tribe Issini seems to demonstrate the considerable karyotypic uniformity, with 2n = 26 + X encountered in all but two of the species studied. This suggests an old and single origin for this karyotype pattern in an ancestor of this tribe. It is worth noting that this chromosome complement matches also a putative ancestral state in the Fulgoroidea as a whole (Kuznetsova et al., 1998; Maryańska-Nadachowska et al., 2006).

The basic chromosome complement of the Issini looks uniform in rough morphology in different species, with similar size differences between chromosomes and gaps, probably the sites of NOR, visible in some cases in the largest pair of autosomes.

Thus the evidence obtained so far by a routine cytogenetic technique allows the suggestion that chromosome evolution did not play an important role in species diversification of the tribe Issini. It is clear however that more advanced techniques are necessary for an adequate cytogenetic characterization of this group.

In the present study, we have characterized the karyotype of two Issini species, *Hysteropterum albaceticum* Dlabola, 1983 and *Agalmatium bilobum* (Fieber, 1877), using silver staining, C-banding and base specific CMA_3- and DAPI-banding, with the aim of identifying possible cytogenetic markers and distinguishing between karyotypes with the same chromosome number 2n = 26 + X. As a result we report here for the first time the distribution and molecular structure of C-heterochromatin regions and the location of nucleolus organizer sites (NORs) in the karyotypes of the family Issidae.

Material and methods

Insects

We used adult males of *Hysteropterum albaceticum* collected in Spain and those of *Agalmatium bilobum* collected in Greece and Italy in 2005 (Maryańska-Nadachowska et al., 2006). Freshly caught specimens were fixed in 3:1 ethanol/acetic acid.

Chromosome preparations

All chromosome preparations were from testes. Each testis consisted of several long fusiform follicles, 10 in *H. albaceticum* while 11 in *A. bilobum*. Follicles were dissected out in a drop of 45% acetic acid and squashed. Then the preparations were frozen on dry ice and, after removal of coverslips, dehydrated in freshly prepared 3:1 ethanol/acetic acid for 20 min and air-dried. The preparations were first analyzed with phase contrast microscope at 400 x. The best chromosome spreads were used for different kinds of staining.

Standard staining

For standard staining the method of Grozeva and Nokkala (1996) was used. The prepara-

tions were first subjected to hydrolysis in 1 N HCl at 60° C for 7 min and stained in Schiff's reagent for 20 min. After rinsing thoroughly in distilled water, the preparations were additionally stained in 4% Giemsa in Sorensen's buffer pH 6.8 for 20 min, rinsed with distilled water, air-dried and mounted in Entellan.

C-banding

For C-banding the method of Sumner (1972) was used. The treatment was carried out using 0.2 N HCl at room temperature for 30 min, a 7-8 min treatment in saturated $Ba(OH)_2$ at room temperature and then a incubation in 2xSSC at 60°C for 1 h. After this, the preparations, finally stained in 4% Giemsa diluted in Sorensen buffer for 10-15 min with excessive staining briefly rinsed in tap water, were mounted in Entellan.

AgNOR-staining

For silver impregnation the method of Howell and Black (1980) was used. Slides were incubated for 6-9 min at 60°C with 60 µl of $AgNO_3$ 0.5 g/ml and 30 µl of a 2% gelatin and 1% formic acid solution. The preparations were rinsed with abundant distilled water and air-dried.

CMA_3- and DAPI-banding

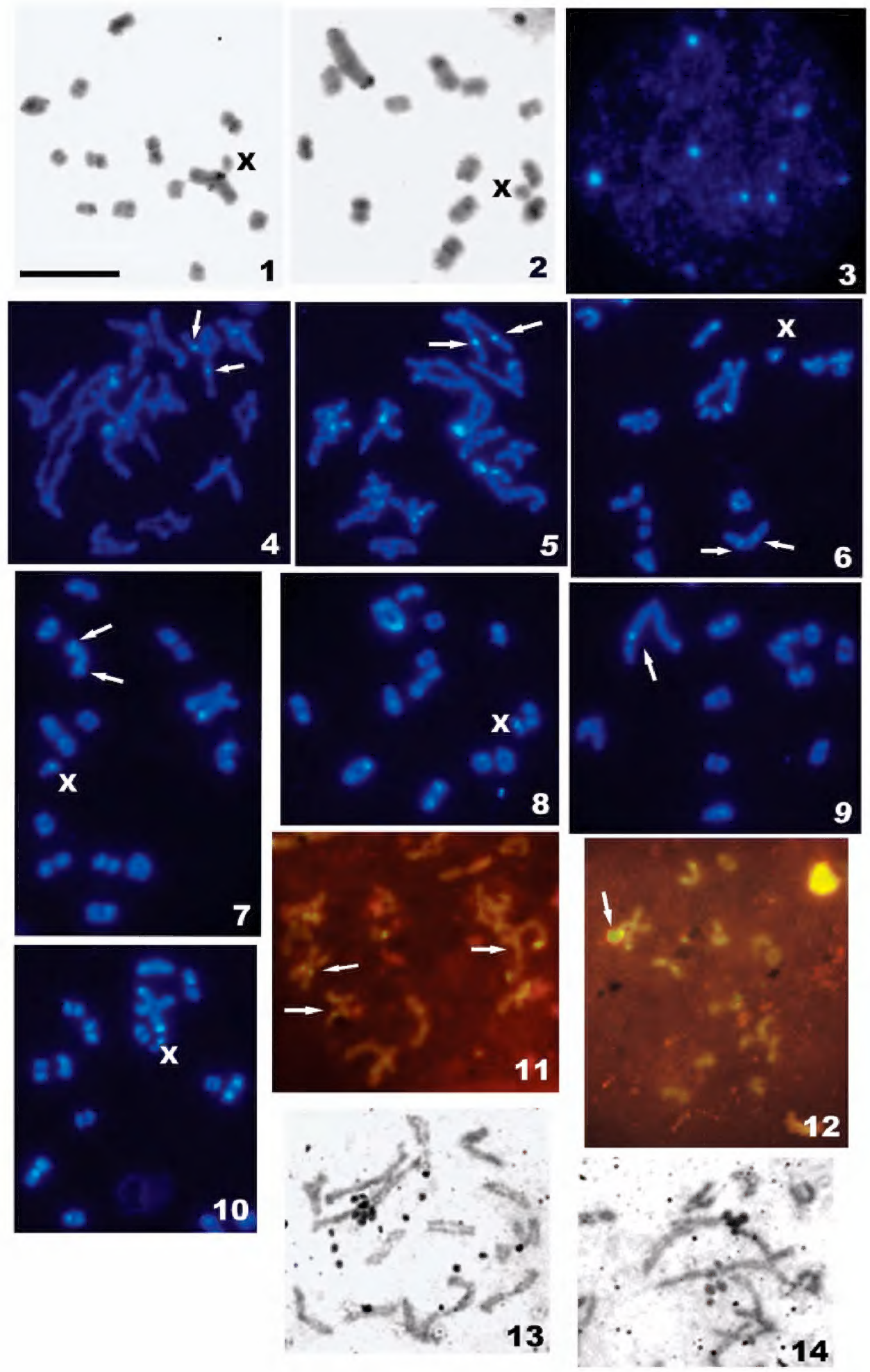
For fluorochrome application the methods of Schweizer (1976) and Danlon and Magenis (1983) were used with minor modifications. C-banded preparations (without Giemsa) were stained first with chromomycin antibiotic CMA_3 (5 µg/ml) for 25 min and then with DAPI (0.4 µg/ml) for 5 min. To improve the fluorochrome staining, 0.5% methanol was included in the staining solutions (Kuznetsova et al., 2001). After staining, the preparations were rinsed in the McIlvaine buffer, pH 7 and mounted in an anti-fade medium (700 µl of glycerol, 300 µl of 10 mM McIlvaine buffer, pH 7, and 10 mg of N-propyl gallate).

All the preparations were analyzed with the aid of a microscope Leica MM 4000 at 1000 x or (the fluorochrome-labeled preparations) a fluorescence microscope Dialux 22 at 1000 x, and the photomicrographs taken using a Camera Nikon DS-U1.

Results

Hysteropterum albaceticum Dlabola, 1983; 2n ♂ = 26 + X

In the present study, the male chromosomal formula of this species, n = 13 + X (sex determination is X0), reported earlier by Maryańska-Nadachowska with co-authors (2006), was confirmed. Unlike the mentioned publication, in which the karyotype was deduced solely from one stage, the spermatocyte metaphase I, we followed a number of consecutive stages of male meiosis and applied the above-listed cytogenetic techniques all allowing an extended characterization of the chromosome complement of this species (Figs 1-14). None of the specimens analyzed showed spermatogonial mitoses. During meiotic stages, 13 bivalents and a univalent X chromosome were observed; one of the bivalents was noticeably larger than the others; the remaining bivalents were gradually decreasing in size; and a rather large X was close in size to the large-sized half-bivalents. Only in a few preparations, C-banding has induced C-blocks, which were found to be present in the majority of bivalents and were of different size and location, both telomeric and interstitial. The largest bivalent exhibited the most conspicuous blocks, which were always located at only one end of every homologue. Furthermore, this bivalent showed a heteromorphy for the size of C-blocks, i.e., they were different in size in the homologues of this pair (Figs 1, 2). This pattern was also confirmed by DAPI staining, which has been operating successfully in this species (Figs 3-



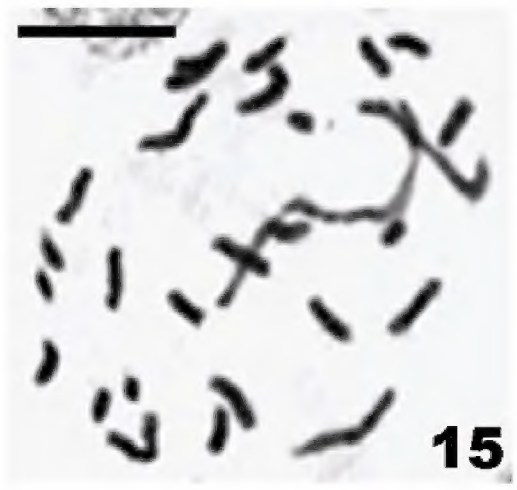
Figs 1-14. Male meiotic chromosomes of *Hysteropterum albaceticum*. **1, 2** - late diakinesis, C-banded; **3** - pre-meiotic interphase, DAPI-staining showing 7 bright and 2 low-power signals; **4-10** - dilpotene (**4**, **5**) and diakinesis (**6-10**), DAPI-stained. The largest bivalent shows one (**4**, **6**, **7**, **9**, **10**), two (**8**) and three (**5**) chiasmata. Arrows point to interstitial signals in the same middle-sized bivalent (**4-7**) and to two interstitial signals in one homologue of the largest bivalent (**9**); **11-12** – diplotene/diakinesis, CMA_3-stained. Arrow point to signals in several bivalents (**11**) and to NOR with a nucleolus in one homologue of the largest bivalent (**12**); **13, 14** - diplotene, silver impregnation showing nucleolar remnants in one (**13**) and both (**14**) homologues of the largest bivalent. Bar = 10 μm.

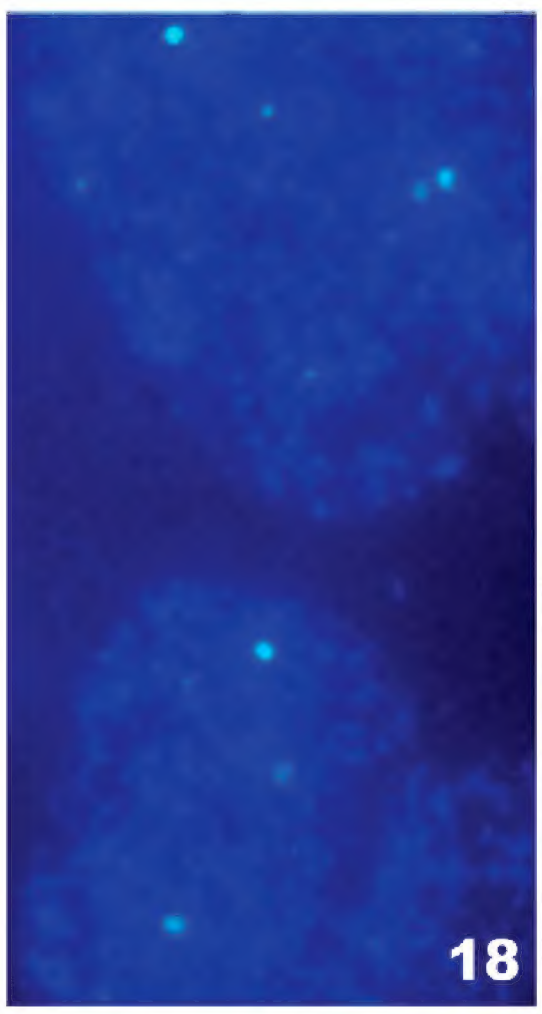
10). Heterochromatin particles were scattered in premeiotic interphase cells and DAPI staining has produced approximately 10-12 fluorescent signals of various sizes and brightness in these cells (7 bright and 2 low-power signals are seen in Fig. 3). This staining also revealed bright signals in the majority of bivalents and in the X chromosome in prophase cells; two or three bivalents only were nearly devoid of heterochromatin (Figs 4-10). During diplotene stage, certain of the bivalents displayed DAPI-positive signals near the end of chromosomes, more often at only one end of a homologue. However in some bivalents, the signals were clearly located interstitially (arrowed in Figs 4, 5, 9). One of these bivalents could be easily recognized in every diplotene and almost every diakinetic (arrowed in Figs 4-7) nucleus due to the presence of a bright interstitial signal in its homologues. The largest bivalent was characteristically marked by bright telomeric signals in both homologues (always present at one end of the chromosome) but in one deplotene/diakinesis cell, one of the homologues exhibited two signals of different size and brightness, both signals having been located interstitially (Fig. 9). The double-stranded X chromosome displayed an interstitial signal in all the nuclei studied. Although CMA_3 -staining was not as successful as DAPI-staining, it has also produced clear signals in the majority of bivalents, including the largest bivalent (Figs 11, 12). Thus, the heterochromatin of this species demonstrated a great affinity to both fluorochromes suggesting that it is equally enriched by AT and GC base pairs. Figure 12 highlights the CMA_3 -treated largest bivalent carrying a NOR with a nucleolus attached at the end of one homologue. Silver staining also showed the presence of silver-impregnated fragmented bodies (nucleolar material) at contact with the largest bivalent, more often with only one (Fig. 13) while sometimes with both (Fig. 14) homologues. This is probably related to the differential activity of the NORs in different homologues. Of interest is however the location of the silver stained nucleolar material clearly at the submedial position rather than at the ends of the chromosomes. The origin of these different patterns, if this is due to an inversion flanking the NOR region or to some other reasons, remained unknown.

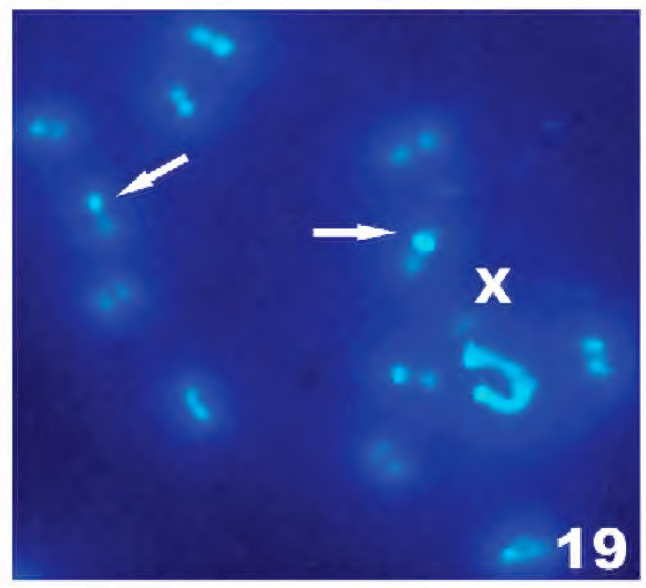
In all the bivalents other than the largest one, there occurred invariably only one crossover point. In diplotene cells, different localization of chiasmata could be seen in the bivalents: terminal or subterminal in some bivalents while clearly interstitial in 3-4 larger bivalents (Figs 4-5). In the largest bivalent, a chiasma was present at different positions, medial, subterminal or terminal, in diplotene/diakinesis cells. In some cells, this bivalent demonstrated the ring shaped formation that is indicative of the occurrence of two chiasmata (Fig. 8), and in one cell three chiasmata were seen in this bivalent (Fig. 5). The location of C-and DAPI-positive signals served to the identification of the chromosome ends, in which a chiasma formed in the largest bivalent and in some other large bivalents. As shown in the Figures presented, in these bivalents the signal could be positioned either at the chromosomal end far from the chiasma or at the chromosomal end near the chiasma. It seems to point to a random chiasma formation in the bivalents. However the analysis of 39 larger bivalents at diplotene/diakinesis stages suggested a kind of preferential association between these patterns: the chiasma was formed at a distance of a heterochromatin block in 28 of these bivalents.

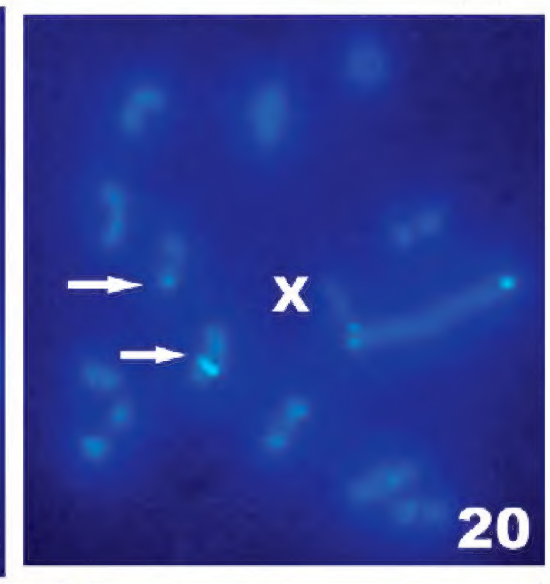
Agalmatium bilobum (Fieber, 1877): 2n♂ = 26 + X

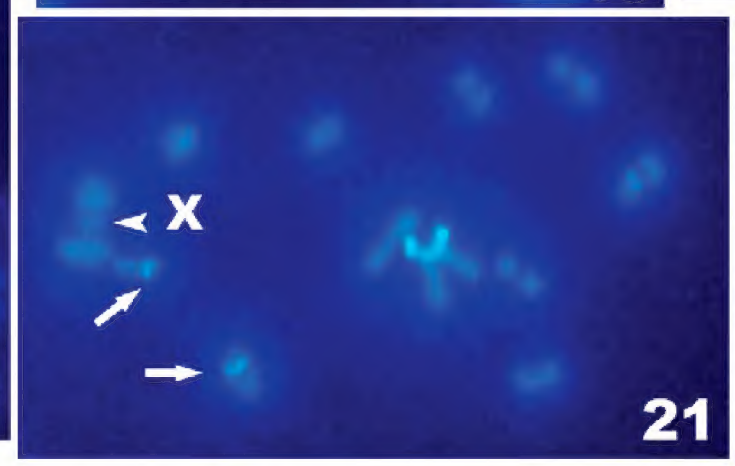
Here, the male chromosomal formula of this species, n = 13 + X (sex determination

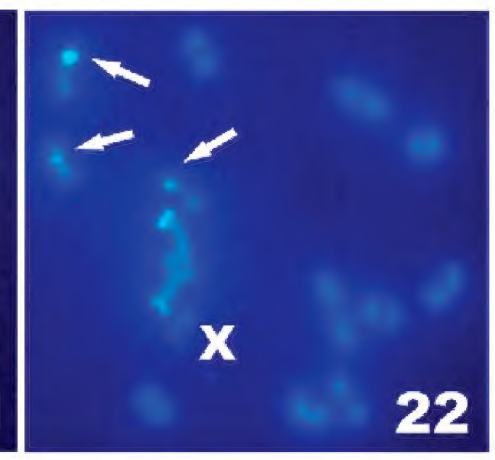


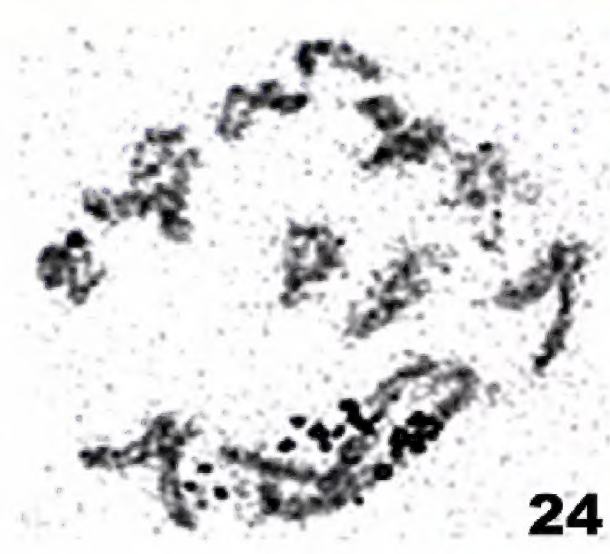












Figs 15-25. Male meiotic chromosomes of *Agalmatium bilobum*. **15** - Spermatogonial prometaphase, C-banded; **16, 17.** Diakinesis, C-banded. **18** - premeiotic interphases, DAPI-staining showing 4 bright and 3 low-power signals (top nucleus). **19-22** – diplotene/diakinesis, DAPI-stained. Arrows point to signals in middle-sized bivalents. **23-25** - Diplotene, silver impregnation showing nucleolar remnants in one (**23**) and both (**24**, **25**) homologues of the largest bivalent. Bar = 10 μm.

is X0), reported earlier by Maryańska-Nadachowska with co-authors (2006), was confirmed. Whereas in the mentioned publication, this karyotype pattern was deduced from MI only, we have studied the chromosome complement of *A. bilobum* in greater detail with the use of mitotic cells as well as meiotic stages other than MI, and cytogenetic techniques such as C-banding (Figs 15-17), DAPI staining (Figs 18-22) and silver impregnation (Figs 23-25). One male exhibited spermatogonial mitoses allowing more precise analysis of chromosome sizes, compared to meiotic pictures, throughout the karyotype. As shown in Fig. 15, there are 27 chromosomes in a spermatogonial prometaphase. All the chromosomes exhibit a well-defined holokinetic structure. By size, we could recognize 5 longer chromosomes, approximately 16 medium-sized chromosomes, and 6 smaller chromosomes. Chromosomes are undistinguishable within the size groups. The first group probably includes an X chromosome; however it could not be distinguished among the chromosomes.

Spermatocyte diplotene/diakinesis cells were made up of 13 bivalents plus the univalent X chromosome (Figs 16, 17, 19-24). During meiotic stages, one bivalent always stands out because of its large size; the remaining bivalents appeared as gradually decreasing in size; and the X chromosome was rather large and close in size to the large-size half-bivalents. C-banding provided conspicuous bands neither in mitotic nor in meiotic chromosomes of this species (Figs 15-17). This result probably suggests not only a methodical failure, but also a small amount of C-heterochromatin in its chromosomes compared to those of *H. albaceticum*. Some of the confirmatory evidence comes from *A. bilobum* premeiotic interphase nuclei, in which usually at most 3-4 bright (plus 3-4 low-power) DAPI-positive signals were visible (Fig. 18), as well as from the following meiotic stages subjected to DAPI-treatment (Figs 19-22). In diplotene/diakinesis nuclei, a number of bivalents showed low-power and, then, not necessarily well visible signals, and only three bivalents, the largest one inclusive, invariably (and sometimes one more; see Fig. 22) showed bright DAPI-positive signals. One of these bivalents was the largest one, which exhibited AT-rich regions at one telomere of one or more often both homologues. As with *H. albaceticum*, the longest bivalent appeared as NOR-bearing with numerous nucleolar remnants arranged in this bivalent at a distance from the midpoint of one (Fig. 23) or both (Figs 24, 25) homologues. Each of the other marker bivalents displayed a brilliant DAPI-positive signal disposed interstitially in one homologue only. All the other bivalents showed either weak or no signals. The X chromosome was DAPI-negative, suggesting that it is deficient of heterochromatin. Our attempts to get CMA_3-banding have failed in *A. bilobum*. The chromosomes of this species are, therefore, suggested to possess mainly AT-rich heterochromatin.

In all diplotene/diakinesis cells investigated in our material, the bivalents, including the largest bivalent, formed each only one chiasma. With the use of DAPI-positive signals as markers of the chromosomal ends we have analysed the location of chiasma in the largest bivalent in different cells. We have found that a single chiasma could be formed in any part of the chromosomes regardless at first glance of the heterochromatin location in this bivalent. However the prevailing pattern found in 10 of 15 diplotene/diakinesis cells investigated was the location of chiasma far from the heterochromatin blocks.

Discussion

Here we have investigated the role of chromosomal bandings in distinguishing be-

tween karyotypes of two planthopper species, *Hysteropterum albaceticum* and *Agalmatium bilobum* (Auchenorrhyncha, Issidae, Issini), exhibiting 2n = 26 +X and a similar general plan of karyotype structure. Previous cytogenetic studies of several dozens of species and genera of the tribe Issini have shown a striking karyotypic uniformity within this group, with 2n = 26 +X found in almost all species investigated (Maryańska-Nadachowska et al., 2006 and references therein; our unpublished data). This karyotype has therefore been proposed as the basic chromosomal complement for the tribe Issini (Maryańska-Nadachowska et al., 2006). It has been generally assumed that holokinetic, or holocentric chromosomes facilitate karyotype evolution by means of dissociations and fusion of chromosomes (White, 1973). Although the data on the tribe Issini are not yet sufficient to allow definite conclusions, it is believed that these rearrangements did not play an important role in species diversification of this group.

Outside the same chromosome number, the chromosomal complement of the tribe Issini, when studied by conventional cytogenetic techniques, was shown to display no detectable inter-species differences in chromosome size and structure. Chromosome banding techniques are known to produce some important landmarks along the chromosomes that allow for the recognition of individual chromosomes within a karyotype and identification of specific regions within individual chromosomes. The identification of chromosomal markers in groups with holokinetic chromosomes is of fundamental importance because of the lack of primary constrictions (the centromeres) in the chromosomes of this type. Consequently rearrangements can not be detected in holokinetic chromosomes unless different banding techniques are used. Despite this, as few as three studies involving chromosomal bandings have so far been published for the Auchenorrhyncha. Perepelov with co-authors (2002) used C-banding to study the karyotypes of *Tibicen bihamatus* and *Platypleura kuroiwae* (the family Cicadidae). The authors found that these phylogenetically remote species, sharing 2n = 18 + X in males, displayed differences in C-heterochromatin amount and distribution: in one species the bands were more abundant and appeared preferentially in the terminal chromosome positions, whereas in the other species they exhibited subterminal and interstitial locations in the chromosomes. Two other publications dealt with the spittlebug species *Phylaenus spumarius* and *Ph. arslani* (the family Aphrophoridae). These phylogenetically related species were shown to be similar in having two pairs of NOR-carrying chromosomes, GC-rich NORs and dot-like C-bands in autosomes while to be different in having distinct karyotypes, 2n = 22 + X and 2n = 18 + neo-XY respectively, and dissimilar sex chromosome C- and DAPI staining patterns (Kuznetsova et al., 2003; Maryańska-Nadachowska et al., 2008).

A male chromosomal complement, 2n = 26 + X, of *H. albaceticum* and *A. bilobum* studied herein agreed with that previously reported for these species by Maryańska-Nadachowska with co-authors (2006). Our karyotype analysis performed with different meiotic stages (as well as spermatogonial mitoses of *A. bilobum*) revealed also that these species are similar in having one pair of large autosome, 12 pairs of medium-sized autosomes gradually decreasing in size, and an X chromosome as one of the larger chromosomes in the set.

NORs

The largest pair of autosomes in *H. albaceticum* and *A. bilobum* appeared to be NOR-bearing as evidenced by Ag-NOR (in both species) and CMA_3 (in *H. albaceticum*)

bandings. NORs are housed interstitially in these chromosomes and this location matches the gaps described previously for *A. bilobum* (Maryańska-Nadachowska et al., 2006). It is common knowledge that the nucleolus usually disaggregates during late prophase and reorganizes at early telophase. During the diplotene/diakinesis stages of both species studied here, nucleolar material (the remnants of argentophilic proteins) was presented as numerous silver-impregnated bodies adjacent to the NOR regions of the chromosomes, the phenomenon repeatedly described in the suborder Heteroptera and known as "the nucleolar fragmentation phenomenon" (Bardella et al., 2008). In *H. albaceticum*, the NOR-related heterochromatin appeared to be essentially GC-rich as became evident after CMA_3-staining. Actually this is true for many other insect groups (Brito et al., 2003; Nechaeva et al., 2004; Severi-Aguiar, 2006; Kuznetsova et al., 2001, 2007; Diego et al., 2008; Criniti et al., 2009) and also for two other Auchenorrhyncha species, *Ph. spumarius* and *Ph. arslani*, studied in this respect (Kuznetsova et al., 2003; Maryańska-Nadachowska et al., 2008). It is worthy of note that in these species, as well as in species studied here, the nucleolus organizer regions are placed in autosomes, but both *Ph. spumarius* and *Ph. arslani* were shown to display two NORs-bearing autosomal pairs.

C-heterochromatin amount, distribution and molecular composition

Numerous discussions of C-banding have emphasized the small amount of constitutive heterochromatin and predominantly terminal or subterminal location of C-blocks in holokinetic chromosomes (Collet et al., 1984; Camacho et al., 1985; Blackman, 1987). However our observations on *H. albaceticum* and *A. bilobum* are clearly contradictory to this statement. Both *H. albaceticum* and *A. bilobum* have shown a great amount of C-heterochromatin, which was however clearly more abundant in *H. albaceticum* chromosomes. In this species, the majority of autosomes and the X-chromosome are marked by conspicuous C-bands of different size and location, terminal, subterminal and quite often interstitial. Only in two or three bivalents, no bands have been detected suggesting that these bivalents are either avoid of heterochromatin or, most likely, contain little heterochromatin. Several bivalents, including the largest one, could be easily identified in meiotic cells as having very characteristic banding patterns. In contrast to what is found in *H. albaceticum*, only the largest and some three other middle-sized bivalents of *A. bilobum* exhibited prominent DAPI-positive C-blocks and demonstrated well distinguishable banding patterns, and no C-bands have been detected in the X chromosome.

Currently some evidence has been advanced which characterizes C-heterochromatin of holokinetic chromosomes with respect to its highly repeated DNA sequences (Bizzaro et al., 2000; Kuznetsova et al., 2003; Grozeva et al., 2006; Lanzone, Souza, 2006; Maryańska-Nadachowska et al., 2008). The results obtained are widely diversified: C-heterochromatin composition may at times demonstrate various patterns of heterogeneity and at other times a kind of homogeneity. In *H. albaceticum*, the heterochromatin has shown a great affinity to DAPI and CMA_3-staining suggesting thus that it is enriched by AT and GC base pairs, i.e. GC-rich clusters are probably dispersed within AT-rich repeats in its heterochromatin. The same is also true for NOR-related heterochromatin, which seems to consist of AT/GC base pair-rich repetitive DNA sequences in this species.

The literature on insect constitutive heterochromatin shows that the knowledge of this type of DNA in insects with holokinetic chromosomes is still fragmented and insufficient.

In recent years however an increasing quantity of such data have appeared and shown that holokinetic chromosomes can display a great deal of C-heterochromatin (Blackman, 1976, 1990; Kuznetsova et al. 1997; Maryańska-Nadachowska, 1999; Vanzela et al., 2000; Grozeva, Nokkala, 2001; Golub et al., 2004; Angus et al., 2004; Pérez et al., 2005; Franco et al., 2006; Bressa et al., 2008; Maryańska-Nadachowska et al., 2008). The species can diverge considerably in this pattern: some display a lot of C-heterochromatin; some show prominent C-bands of different size in separate chromosomes; and in other species C-heterochromatin appeared to avoid detection due to its presence in small or minute amounts. Actually, this happens also with monocentric groups, in which some species are known to have a large amount of C-heterochromatin but some species not (King, John, 1980; Hoshiba, Imai, 1993; Graphodatsky, Fokin, 1993; Swarça et al., 2003; Rozek et al., 2004). This allows suggestion that holokinetic chromosomes in fact differ slightly or not at all in constitutive heterochromatin amount from monocentric chromosomes. The heterochromatin in both holokinetic and monocentric chromosomes is known to share a similar molecular composition being principally composed of the satellite DNAs (Mandrioli et al., 2003). These chromosomes are however different in C-heterochromatin distribution patterns. Monocentric chromosomes are known to have pericentromeric and telomeric C-bands while very rarely interstitial bands. Holokinetic chromosomes display no centromeres and thus lack pericentromeric heterochromatin, but telomeric and interstitial C-blocks are clearly characteristic of these chromosomes. Thus, a hypothesis of "equilocal distribution of heterochromatin", i.e. the tendency of heterochromatin to be located at similar positions along nonhomologous chromosomes (Heitz, 1933) typical for monocentric chromosomes (Schweizer, Loidl, 1987), is not thus valid for holokinetic chromosomes (Mandrioli et al., 2003).

Chiasmata

H. albaceticum and *A. bilobum* showed chromosomal behavior during spermatocyte meiosis similar to that already reported in other auchenorrhynchan species (Halkka, 1964; Kuznetsova et al., 1998, 2009). The bivalents most often had a single chiasma but the larger bivalents could sometimes display two chiasmata, and the largest bivalent has shown three chiasmata in one cell. The low number of chiasmata (estimated to be 1-2 from cytological analyses) seems to be a standard pattern in holokinetic bivalents (Halkka, 1964). Although the bivalents with multiple chiasmata (up to four, inclusive) have been observed in holokinetic groups, including the Auchenorrhyncha (Kuznetsova et al., 2003, 2009; Maryańska-Nadachowska et al., 2008; present paper), these observations never advanced beyond the metaphase I of spermatogenesis and the further fate of cells with multichiasmatic bivalents remained therefore unknown. Nokkala with co-authors (2004) have provided a detailed analysis of chiasma formation and distribution in holokinetic bivalents of the psyllid species *Beopelma foersteri* (Flor, 1861) (Homoptera, Sternorrhyncha, Psylloidea). These authors showed that more than two chiasmata in a holokinetic bivalent will obstruct the regular course of meiosis and result in the elimination of cells of this type, providing a strong selection against formation more than two chiasmata in holokinetic bivalents.

Heterochromatin is known to suppress recombination of chromosomal segments in its vicinity; this being so, crossing-over rarely occurs in heterochromatic regions (John, King, 1985; Sumner, 1990). We have showed that when only one chiasma was formed in the larg-

er bivalents of *H. albaceticum* and *A. bilobum*, the chiasma could have medial, subterminal or terminal positions in these bivalents in neighbouring cells. Using C/DAPI-positive signals as markers of the chromosome ends, we have analysed the chiasma position variation in the larger bivalents from both species according to the heterochromatin distribution per bivalent. We have found that a chiasma could be positioned either at the chromosomal end far from C-heterochromatin block or at the chromosomal end adjacent to C-block. It seemed to point to a random chiasma formation in the bivalents. However the aggregate (in both species) analysis of 54 bivalents in diplotene/diakinesis cells revealed a kind of preferential association between these patterns: in 72% of the bivalents analysed a chiasma was formed at a distance of C-heterochromatin blocks. Quite recently, Bressa and co-authors (2008) have published similar and even more statistically plausible results for a bug species *Holhymenia rubiginosa* Breddin, 1904 (Heteroptera: Coreidae). In this species, the chiasmata were found to occur at any position with reference to C-heterochromatin blocks; however in 87% of 203 bivalents investigated a chiasma was housed at a point remote from C- blocks.

Heteromorphism

One further observation which clearly needs to be discussed here concerns the frequency of heteromorphisms found in *H. albaceticum* and *A. bilobum* meiosis. These are heteromorhism for NORs of an absence/presence type and that for the presence/absence, size and number of C-heterochromatin blocks (see Results). However speculations on possible explanations would call for more detailed analysis of these patterns that was beyond the scope of our present work.

Conclusions

The main implication of this study is the following. So far it has been assumed that the auchenorrhynchan tribe Issini is characterized by highly uniform karyotypes giving no way of distinguishing between the species and inferring the chromosome evolution in the whole group.

Our results showed that even if *H. albaceticum* and *A. bilobum* have karyotypes with the same chromosome number and a similar general plan of structure, they differ considerably in banding patterns; the differences being such as to indicate that many intrachromosomal rearrangements (although other rearrangements have also been involved) are responsible. Moreover, the chromosome banding techniques applied have revealed the discriminatory landmarks in the chromosomes and provided evidence for the existence of marked and variable chromosomal polymorphism within the studied populations of these species.

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